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Abstract

Background:

PRIMVAC is a VAR2CSA-derived placental malaria (PM) vaccine candidate aiming to prevent serious clinical outcomes of *Plasmodium falciparum* infection during pregnancy. In this phase Ia/Ib clinical trial, we assessed the safety and immunogenicity of PRIMVAC adjuvanted with Alhydrogel® or Glucopyranosyl Lipid Adjuvant in stable emulsion (GLA-SE) in non-pregnant French and Burkinabe women.

Methods:

This first-in-human randomised, double-blind, placebo-controlled, dose escalation trial was conducted in two staggered phases, a phase Ia in 18-35 years old malaria naïve women in France and a subsequent phase Ib in malaria exposed nulligravid women in Burkina Faso. Volunteers were recruited into four sequential cohorts receiving PRIMVAC intra-muscularly at day 0 (D0), day 28 (D28) and day 56 (D56): two in France receiving 20 µg or 50 µg of PRIMVAC and then two in Burkina-Faso receiving 50 µg or 100 µg of PRIMVAC. Within each cohort, volunteers were randomised to two arms (PRIMVAC adjuvanted with either Alhydrogel® or GLA-SE) in France and three arms (PRIMVAC adjuvanted with either Alhydrogel® or GLA-SE or placebo) in Burkina Faso. The primary endpoint of the study was the proportion of participants with any grade 3 or higher adverse reaction to vaccination until day 35. Safety at later time points as well as humoral and cellular immunogenicity were assessed in secondary endpoints. This trial is registered with ClinicalTrials.gov, number NCT02658253.

Findings:

Between April 19 2016, and July 13 2017, a total of 68 women (18 in France, 50 in Burkina Faso) were included. No serious adverse event following immunization (SAEFI) related to the

vaccine occurred. PRIMVAC antibody titres increased with each dose and seroconversion was observed in all the PRIMVAC vaccinated women (N=57). PRIMVAC antibody titres reached a peak (geometric mean 11843·0 OD 1·0, 95% CI 7559·8-18552·9 with 100 µg dose and GLA-SE) one week after the third vaccination (Day 63). In comparison to Alhydrogel®, GLA-SE tended to improve the PRIMVAC antibody response (geometric mean 2163·5 OD 1·0, 95% CI 1315·7-3557·7 with 100 µg dose and Alhydrogel at Day 63). One year after the last vaccination, 20 out of 28 (71·4%) PRIMVAC/Alhydrogel® and 26 out of 28 (92·9%) PRIMVAC/GLA-SE vaccinated women still had PRIMVAC antibodies, although antibody magnitude was markedly lower (452·4 OD 1·0, 95%CI 321·8-636·1 with 100 µg dose and GLA-SE). These antibodies reacted with native homologous VAR2CSA expressed by NF54-CSA infected erythrocytes (IEs) (fold change from baseline at Day 63 with 100 µg dose and GLA-SE: 10·74, 95% CI 8·36-13·79). Limited cross-recognition, restricted to sera collected from women that have received the 100 µg PRIMVAC dosage, was observed against heterologous VAR2CSA variants expressed by FCR3-CSA and 7G8-CSA IEs (fold change from baseline at Day 63 1·49, 95%CI 1·19-1·88 and 1·2, 95% CI 1·08-1·34, respectively). PRIMVAC antibodies also inhibit the interactions between the homologous VAR2CSA expressed on NF54-CSA IEs and its placental receptor, chondroitin sulfate A (CSA) (% inhibition from baseline at Day 63 with 100 µg dose and GLA-SE: 58.3, 95% CI 48·5-68·1).

Interpretation:

PRIMVAC adjuvanted with Alhydrogel® or GLA-SE had an acceptable safety profile, was immunogenic and induced functional antibodies reacting with the homologous VAR2CSA variant expressed by NF54-CSA IEs. Limited cross-reactivity against heterologous VAR2CSA variants was only observed in the higher dosage group. Other schedule of immunization,

antigen dosage and combinations with other VAR2CSA-based vaccines are envisaged to improve the cross-reactivity.

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Keywords: Malaria, *Plasmodium*, vaccine, VAR2CSA, pregnancy, placenta, First-in-Human, clinical trial.

Research in context

Evidence before this study

We systematically searched PubMed on June 4, 2019, for articles in English investigating the relationship between VAR2CSA and placental malaria (PM), using the terms ("VAR2CSA" [All Fields] AND "pregnancy" [All Fields] AND "malaria" [All Fields]) and also VAR2CSA and vaccine, using the terms ("VAR2CSA" [All Fields] AND "vaccine" [All Fields]). Our search returned respectively 182 and 106 articles, clearly indicative of a link between VAR2CSA and PM vaccine development. We also searched for articles about clinical trials of PM vaccines. We searched using the terms ("malaria" [All Fields] AND "vaccines" AND "placenta" [All Fields] AND "clinical trial" [All Fields]) OR ("malaria" [All Fields] AND "vaccines" AND "pregnancy" [All Fields] AND "clinical trial" [All Fields]) OR ("placental malaria vaccine" [All Fields] AND "clinical trial" [All Fields]). Only one recently published phase Ia study reporting the safety and immunogenicity of a VAR2CSA-derived placental malaria vaccine in malaria-naïve volunteers in Germany was identified. No clinical trial reporting the safety and immunogenicity of a placental malaria vaccine in a malaria endemic country was found.

Added value of this study

This is the first report of the safety and immunogenicity of a VAR2CSA-derived PM vaccine in both malaria naïve and *P. falciparum*-exposed non-pregnant women. PRIMVAC adjuvanted with either Alhydrogel® or GLA-SE delivered by intramuscular (IM) injection had an acceptable safety profile. All women vaccinated with PRIMVAC seroconverted after two vaccine doses and a high proportion of them were still seroconverted one year after the last immunization. Furthermore, PRIMVAC generated antibodies able to react with native VAR2CSA expressed on the surface of different strains and also inhibited the adhesion of the

homologous NF54-CSA strain to CSA. Limited cross-recognition, restricted to sera collected from women that have received the 100 µg PRIMVAC dosage, was observed against the FCR3-CSA and 7G8-CSA VAR2CSA-expressing parasites.

Implications of the available evidence

The findings from this first-in-human clinical evaluation of the PRIMVAC vaccine in women in France and in Burkina Faso lays the foundation for further clinical evaluation. The advanced development of this PM vaccine candidate, alone or in combination with other PM vaccine candidates, now requires a deeper characterization of the breadth of vaccine-induced immune responses (humoral and cellular) upon natural exposure during pregnancy.

INTRODUCTION

According to the latest WHO's Malaria Report, 219 million cases of malaria occurred in 2017 leading to 435 000 deaths.[1] The majority of clinical cases and deaths occurred in sub-Saharan Africa and were mainly resulting from *Plasmodium falciparum* infection. In malaria endemic areas, individuals progressively acquire clinical immunity during childhood and adults are therefore generally protected against the severe clinical outcomes of the disease.[2] However, during their first pregnancies, women become once again susceptible to the serious clinical outcomes associated with placental malaria (PM).[3] PM can lead to maternal anaemia, hypertension as well as stillbirth and low birth weight (LBW) due to premature delivery and foetal growth retardation.[3] LBW is a significant risk factor for neonatal and infant death.[4] A modelling study showed that up to 40% of pregnant women in sub-Saharan Africa develop PM.[5] Furthermore, *P. falciparum* malaria was responsible for 11% of LBW-related infant mortality in Sub-Saharan Africa[6] and an estimate of 217 026 stillbirths (20% of all stillbirths in sub-Saharan Africa).[7] Remarkably, the prevalence of PM sharply drops with successive pregnancies.[4] This protection has been associated with the development of antibodies directed towards the surface of infected erythrocytes (IEs) from placental origin.[8] Therefore, a vaccine priming the immunity observed in multigravid women could have a high impact on both disease incidence and severity and then save hundred thousand lives each year.[3]

The severe outcomes of PM results from the massive accumulation of IEs in the placental intervillous spaces [2] through the binding to the placental CSA, a binding phenotype not seen outside of the pregnancy context.[9] IEs adhesion is mediated by the highly diverse *P. falciparum* erythrocyte membrane protein family (PfEMP1) encoded by the *var* genes.[2]

Evidences strongly support the VAR2CSA-PfEMP1 variant as the leading candidate for a PM vaccine. Indeed, VAR2CSA is preferentially expressed by IEs from placental origin[10] and recombinant VAR2CSA binds to CSA.[11] Genetic deletion of *var2csa* results in the loss of IEs

adhesion to CSA that cannot be compensated by any other PfEMP1.[12] Anti-sera to recombinant VAR2CSA react to the surface of CSA-binding IEs and inhibit their adhesion to CSA[13-15]. Women gradually acquire strain-transcendent antibodies recognizing recombinant and native VAR2CSA expressed on IEs that also inhibit IEs adhesion to the placenta, thus correlating with PM protection.[8,16,17] Taken together, these data provide a rational basis for developing a VAR2CSA-derived PM vaccine. Two PM vaccine candidates (PRIMVAC and PAMVAC) are currently under clinical development.[18] Following an extensive screening study, PRIMVAC spanning the CSA-binding DBL1x-2x region of the 3D7-VAR2CSA variant was down-selected[15] and transitioned to current good manufacturing practice (cGMP) production.[19] Preliminary safety and immunogenicity results of the PAMVAC study, assessing a PM vaccine derived from the FCR3-VAR2CSA variant in malaria naïve volunteers in Germany, reported that PAMVAC is safe and can induce functional antibodies against the homologous VAR2CSA-expressing strain.[20]

Here, we report the safety and immunogenicity of PRIMVAC in both malaria naïve non-pregnant women in France and in *P. falciparum*-naturally exposed nulligravid women in Burkina Faso.

METHODS

Study design and Participants

This is a first-in-human phase Ia/Ib dose escalation trial, evaluating the safety and immunogenicity of three vaccinations with progressively higher dosages of PRIMVAC adjuvanted with Alhydrogel® or GLA-SE. A total of 68 healthy adult non-pregnant women were enrolled in four sequential cohorts. The trial started with two cohorts in malaria-naïve women in France (phase Ia, with 20 µg of PRIMVAC in cohort A, and then 50 µg of PRIMVAC in cohort B) and then pursued with two cohorts in nulligravid women living in Burkina-Faso (phase Ib, with 50 µg of PRIMVAC in cohort C, and then 100 µg of PRIMVAC in cohort D). Within each cohort, volunteers were randomized in a double-blind manner to two arms (PRIMVAC adjuvanted either with Alhydrogel® or GLA-SE) in phase Ia, and to three arms (PRIMVAC adjuvanted either with Alhydrogel® or GLA-SE or placebo) in phase Ib (**Table S1; appendix p10**). There was no placebo control arm in phase Ia. Enrolment into each cohort opened progressively following pre-defined rules after interim review of safety data and advise by an independent Data Safety Monitoring Board (DSMB). Full details of the study design are provided in the appendix pp 3-6. The protocol (N° EudraCT: 2015-002246-31) was approved by the French national Ethics Committee "CPP Ile de France III" (recorded under n°3328) and the French Medicine Agency (ANSM) (recorded under n°151347A-61) in France, and by the National Ethics Committee (CERS), the Institutional Bioethics Committee of the CNRFP (CIB-CNRFP) and the National Regulatory Authority (CTEC) in Burkina Faso. The trial was registered on ClinicalTrials.gov under the number NCT02658253 and was conducted in accordance with the Helsinki declaration.

Healthy non-pregnant women aged 18-35 years without any history of malaria and without recent travel or travel plans to malaria-endemic regions were eligible for enrolment in France. In Burkina Faso, healthy nulligest women aged 18-35 years were eligible, without any

restrictions with regards to prior *Plasmodium* infections or exposure. Volunteers were not eligible if they had previously received investigational malaria vaccines or if they intended to become pregnant during the trial. All participants provided written informed consent. Detailed eligibility criteria are provided in the appendix pp 3-5.

Randomisation and masking

French participants were randomized in a 1:1 ratio to receive PRIMVAC adjuvanted with Alhydrogel® or GLA-SE. In Burkina Faso, participants were randomized in a ratio of 1:2:2 to receive either placebo or PRIMVAC adjuvanted with Alhydrogel® or GLA-SE, respectively. The randomization sequence, using stratification by cohort and blocks of variable size, was computer generated with SAS software (version 9·3) by the unblinded statistician (EUCLID/F-CRIN Clinical Trials Platform, CIC1401, Bordeaux, France) and implemented in a validated web-based randomization tool (Clinsight® software). The participants were randomized by the investigator on their first vaccination visit (D0). Upon randomization, the Clinsight® software allocated the blinded treatment number via the electronic Case Report Form (eCRF). The correspondence list was made available at each site's pharmacy, where the unblinded pharmacist prepared and dispensed the masked vaccine syringe. All other site's staff and participants were masked to the treatment assignment.

Procedures

Vaccine candidate

PRIMVAC expressed in *E.coli* SHuffle® strain was manufactured by Novasep Henogen (Gosselies, Belgium) on behalf of Inserm as the legal sponsor.[19] According to the randomized arm, volunteers received at days 0, 28 and 56, three intra-muscular (IM) injections of 20 µg, 50 µg or 100 µg of PRIMVAC adjuvanted either with Alhydrogel® (0.85 mg aluminium content

per injection), manufactured by Brenntag (Denmark) or with GLA-SE (a stable oil-in-water emulsion containing glucopyranosyl lipid A (GLA), an investigational medicinal product (IMP) developed by the Infectious Disease Research Institute (IDRI, USA)).[21]

GLA-SE was administered at 2·5 μg GLA in 2% oil per vaccine injection for cohorts receiving 20 μg or 50 μg of PRIMVAC or at 2·56 μg GLA in 2% oil per vaccine injection for cohorts receiving 100 μg of PRIMVAC. Volunteers randomized to placebo in phase Ib received saline solution (NaCl 0·9%) (Chaix et du Marais, France). Preparation of the IMPs (PRIMVAC adjuvanted with Alhydrogel® or GLA-SE, or placebo) were performed by the pharmacist or an approved collaborator of the clinical site and delivered to the site study nurse or investigator in an individual masked syringe.

Follow up

In France, the volunteers were contacted by phone for the assessment of safety the day after each vaccination as well as 14 days later. To collect the adverse events following immunization (AEFI) in Burkina Faso, trained nurses under the supervision of the study clinicians paid a daily home visit to each enrolled study participant from days 1 to 7 after each vaccination. On-site safety visits took place seven days after each vaccination (at days 7, 35, and 63) and additional follow-up visits at days 90, 180 and 421. At each on-site visit, blood samples were collected for safety lab assessments at the local laboratories as well as for storage for later immunogenicity measurements. Between visits, volunteers recorded solicited local and systemic adverse events and any other symptoms in a diary booklet. Clinical data were collected and entered in the eCRF on site. Trial pausing rules were defined in the protocol in case of safety concerns **(appendix; p 6)**.

Immune response

PRIMVAC total IgG antibody and isotypic subtypes antibody titres were measured by enzyme-linked immunosorbent assay (ELISA) **(appendix p8)**. Data were fit to a 4-parameter sigmoidal curve, and the reciprocal serum dilution at which the optical density was 1·0 (OD1·0) for total IgG or 0·5 (OD0·5) for isotypic subtypes was calculated. Samples were considered positive if the difference between the post-immunization optical density (OD) 1·0 and the pre-immunization OD1·0 (net OD1·0) was >50 and the ratio of post-immunization OD1·0 to pre-immunization OD1·0 (ratio) was >2·5 as previously described.[22]

The cellular immune response was assessed *in vitro* by measuring production of the T-cell IL-2 and IL-5 cytokines by ELISPOT (Diaclone) after stimulation with the VAR2CSA antigen at a concentration of 10 μg/ml **(appendix pp 8-9)**. A response was considered positive if the number of spots in the wells stimulated with the vaccine antigen was twofold higher than the number of spots in the negative control using a cut-off of 10 spot forming cells (SFC)/10^5 cells after background subtraction. In addition, the experiment was considered to be interpretable if the ELISPOT response to PMA-Ionomycin was ≥ 100 SFC for IL-2 and ≥ 10 SFC for IL-5, respectively.

P. falciparum laboratory adapted parasite strains NF54, FCR3 and 7G8 were grown and selected for the CSA-binding phenotype. These selected populations are referred to as NF54-CSA, FCR3-CSA and 7G8-CSA. Sera were assessed for reactivity against native VAR2CSA variants expressed on the surface of NF54-CSA, FCR3-CSA and 7G8-CSA IEs as previously described[15] using 50 μl of sera diluted (1:2) in PBS 1% BSA **(appendix p 9)**. Results were expressed as the ratio of the geometrical mean fluorescence intensities (Geo. MFI) in the PE channel of the post-immunization samples over the respective pre-immunization samples.

Inhibition of IEs binding to CSA by sera collected at days 0, 35, 63 and 90 (1:5 dilution) previously decomplemented at 56°C for 45 minutes was assessed in a 96-well plate high throughput format as previously described[19]. Results were expressed as % of inhibition of the post-immunization samples compared to the respective pre-immunization samples [% inhibition = $100-(OD_{post\text{-}immunization}/OD_{pre\text{-}immunization})/100$].

Outcomes

The primary objective was to assess the safety of repeated PRIMVAC administration. The primary safety endpoint, also considered as the dose-escalation criterion by the DSMB, was the proportion of participants per cohort and randomized arm with any grade 3 and persisting at grade 3 for > 48 hours or higher clinical or laboratory adverse reaction to vaccination reported by the site investigator between day 0 and day 35. An adverse reaction was defined as an adverse event (AE) following immunization (AEFI) considered to be related or possibly related to the vaccine by the site investigator. AE were graded with the INYVAX EC FP7 Brighton Collaboration Foundation grading scale.[23] All events not covered by this scale were graded using the FDA scale, which was adapted for Burkina Faso taking into account the local reference ranges **(Table S2; appendix p 11)**. Secondary safety endpoints included the number and proportion of AE and serious AE (SAE) at various time points after vaccination.

The secondary objective was to assess the PRIMVAC immunogenicity through 1) the humoral immune response (total IgG at D0, D28, D35, D56, D63, D90, D180 and D421 and isotypic subtypes at D0 and D90) and 2) the cellular immune responses after *in vitro* stimulation with the vaccine antigens (IL5 and IL2 secreting T cells) at D0, D7 and D63. Description of a binary response variable for seroconversion was added after initial description of the quantitative titres. Exploratory immunogenicity endpoints, measured at D0, D35, D63 and D90, concerned the capacity of the specific vaccine antibodies (IgG) to 1) recognize different VAR2CSA variants

expressed on the surface of parasitized erythrocytes and 2) to inhibit interactions between parasitized erythrocytes expressing different VAR2CSA variants and CSA.

Statistical analysis

Acknowledging that due to the small sample size, the power of phase I trials is in general very low, 10 participants per active arm in cohorts C and D constitute a trade-off between detectable event rate and power in the context of a phase I trial.[24] A sample size of 10 participants per active cohort arm in cohorts C and D allowed the observation of at least one SAE with 80% power if the underlying SAE rate is at least 15% **(appendix pp 6-8)**.

Statistical analyses were performed per dose cohort and randomized arm and for the pooled placebo arms of cohorts C and D. A modified intention to treat (mITT) approach, including all participants having received at least one injection was used for the primary statistical analyses. Per protocol analyses were performed as additional analyses **(appendix pp 25-29)**.

Proportions of the primary endpoint were described with 90% two-sided binomial confidence intervals. Geometric means and their two-sided 95% confidence interval were used to describe antibody titres. To evaluate the magnitude of the humoral (total IgG) and cellular responses (IL2 and IL5 secreting T cells), intra-group comparisons were performed using the Wilcoxon paired signed-rank test with a two-sided significance level of 5%. No statistical comparisons between groups or cohorts were done as this phase I trial was not designed for such comparisons. Interim safety reviews by the independent DSMB prior to dose escalation were based on descriptive analyses. All analyses were performed using SAS software (version 9·4).

Role of the funding source

Inserm was the sponsor of the study. This study has been funded by the European Vaccine Initiative (EVI) (via funds from German Ministry for Education and Research (BMBF) through

Kreditanstalt für Wiederaufbau (KfW) and Irish Aid, Department of Foreign Affairs and Trade, Ireland), Inserm and Institut National de Transfusion Sanguine. EVI and Inserm representatives were members of the Trial Steering Committee and as such were involved in the study design, the overview of the study conducts and analyses, the writing of the report and in the decision to submit the manuscript for publication.

RESULTS

Between April 19, and July 5, 2016, 25 women were screened and 18 were included in cohorts A and B in France **(Figure 1)**. Six participants were first assigned to receive 20 µg of PRIMVAC in combination with either Alhydrogel® (n=3) or GLA-SE (n=3). The 12 other participants were assigned to receive 50 µg of PRIMVAC/Alhydrogel® (n=6) or 50 µg of PRIMVAC/GLA-SE (n=6). Between November 25, 2016 and July 13, 2017, 76 women were screened and 50 were included in cohorts C and D in Burkina Faso (**Figure 1**). Of the 49 women screened for cohort C, 25 were included and randomized to receive 50 µg dosage of PRIMVAC/Alhydrogel (n=10) or PRIMVAC/GLA-SE (n=10) or placebo (n=5). Of the 27 women screened for cohort D, 25 were included in each cohort and randomized to receive 100 µg dosage of PRIMVAC/Alhydrogel (n=10) or PRIMVAC/GLA-SE (n=10) or placebo (n=5). The main characteristics of the enrolled participants in each cohort and randomized arm are described in **Table 1**.

During the trial, after review of the safety data, the DSMB recommended opening of the cohorts as planned by the protocol. Pregnancy of one woman in cohort B was discovered immediately after randomization and she thus did not receive any injection and was excluded from the mITT analyses. Among the 67 volunteers included in the analyses, 63 received all three injections. In cohort C, due to a pregnancy, one woman in the GLA-SE group did not receive the third vaccination. In cohort D, one woman in the placebo group and one woman in the GLA-SE group did not receive the third vaccination and one woman in the placebo group did not receive the second and the third vaccinations (withdrawal of consent).

Participants who received PRIMVAC at all dosage and adjuvant combinations had a good safety profile, with most AE being of grade 1 or grade 2 **(Table 2 and Table S3; appendix pp 12-13)**. No grade 3 or grade 4 clinical or biological AE related to the vaccine was observed during the trial. A total of 338 AE and 186 adverse events following immunization (AEFI)

related to the vaccine were documented **(Table 2 and Table S3; appendix pp 12-13).** For the cohort A (n=6), 54 AE were reported, 45 related to the vaccine: 25 in the Alhydrogel® group (grade 1[n=20]; grade 2[n=5]) and 20 in the GLA-SE group (grade 1[n=16]; grade 2 [n=4]). For the cohort B (n=11), 105 AE were reported, 84 related to the vaccine (82 clinical and 2 biological): 40 clinical AE in the Alhydrogel® group (grade 1 [n=21]; grade 2 [n=19]) and 42 in the GLA-SE group (grade 1[n=28]; grade 2 [n=14]). Two SAE (meningeal syndrome grade 3 n=1 and anemia grade 3 n=1) were reported in the GLA-SE group of cohort B, 7.5 and 14 months respectively after the last vaccine injection and were considered not related to vaccination. For the cohort C (n=25), 94 AE were reported, 33 related to the vaccine: 18 in the Alhydrogel® group (grade 1[n=14] or 2 [n=4]), and 10 in the GLA-SE group (grade 1 [n=9] or 2 [n=1]) and 5 in the placebo group (grade 1 [n=5]). For the cohort D (n=25), 85 AE were reported, 24 related to the vaccine: 15 in Alhydrogel® group (grade 1[n=15]) and 9 in the GLA-SE group (grade 1[n=8] or 2 [n=1]). Thirty cases of serious biological AE (hyponatremia [n=27], hypoprotidemia [n=1] and hyperkalaemia [n=2]), were reported in Burkina Faso (cohort C and D). None were related to the vaccine or to the study procedure. Hyponatremia events were attributed to measurement errors due to a malfunction of the electrolyte analyzer. No adverse pregnancy outcomes have been reported for the pregnancies occurring in trial participants.

The most common vaccine related AE (N=186) were injection site-related events (n=123 [66·1%]), asthenia (n=19 [10·2%]), and headache (n=18[9·7%]). The most frequent local reactions were pain at injection site and limitation of arm motion abduction **(Table 3)**.

The primary safety endpoint (proportion of participants per cohort with any grade 3 and persisting at grade 3 for > 48 hours or higher clinical or laboratory adverse reaction to vaccination reported by the site investigator between day 0 and day 35) was 0% in all cohorts and arms, except for the pooled placebo arm, in which this proportion was estimated at 10%

(N=10) (**Table S4; appendix p 14)**. This was not due to an observed adverse event but to the fact that the endpoint observation was missing for one volunteer who withdrew her consent prior to day 35 and who as imputed as a safety "failure" in the statistical analysis (analysis strategy missing=failure).

Antibodies titres to PRIMVAC were assessed by ELISA on sera collected on vaccinations days (D0, D28 and D56), seven days after the second and third vaccinations (D35 and D63) as well as 90, 180 and 421 days after the first vaccination. No PRIMVAC antibody titre above 100 was detected in any of the volunteers in cohorts A and B before vaccination. Sixteen women from cohorts C and D had detectable levels of antibodies before vaccination (dilution above 1/100 to reach an OD of 1·0) (maximum geometric mean titre encountered at baseline: 32·6 OD1·0 [95%CI 6·7-158·6]) **(Figure 2 and Table S5; appendix p15, Figure S1; appendix p22)**. Antibody titres to PRIMVAC increased with each dose with the highest levels usually observed 7 days after the third vaccination (D63) **(Figure 2 and Table S5; appendix p 15, Figure S1; appendix p22)**. Placebo recipients (cohorts C and D) did not have any marked antibody increases over time. Positive antibody responses were observed in 56 out of 57 PRIMVAC-vaccinated women 28 days after the second injection (D56) **(Table S6; appendix p 16)**. Seroconversion could not be assessed at D56 and D63 for one volunteer (cohort D GLA-SE) who did not come to the planned visits and then did not receive the third vaccination. Interestingly, although having received only two vaccinations, she had a positive antibody response at D90 and up to the last visit (D421). A trend for higher geometrical means was observed for all the cohorts vaccinated with PRIMVAC adjuvanted with GLA-SE compared to Alhydrogel® (geometric means at D63, 5997·7 OD 1.0 [95% CI 4208·9-8546·6] and 1581·7 [95% CI 925·0-2 704·4] respectively for cohort C PRIMVAC 50µg; 11843·0 [95% CI 7 559·8-18552·9] and 2163·5 [95% CI 1 315·7-3 557·7] respectively for cohort D PRIMVAC 100µg)

(Figure 2 and Table S5; appendix p 15 and Figure S1; appendix p22). The highest antibody level was reached one week after the third vaccination (Day 63) in the 100 µg PRIMVAC/GLA-SE cohort (geometric mean 11843 OD 1·0, 95% CI 7559·8-18552·9). Antibody titres decreased gradually in all PRIMVAC-vaccinated women between D63 and D421. Four months (D180) after the last vaccination, 54 out of the 56 (96·4%) PRIMVAC-vaccinated women still had positive PRIMVAC specific antibody responses, while one-year (D421) after the last vaccination, 20 out of 28 (71·4%) PRIMVAC/Alhydrogel® and 26 out of 28 (92·8%) PRIMVAC/GLA-SE vaccinated women still had positive responses **(Table S6; appendix p 16)**. Although two volunteers of the higher PRIMVAC/GLA-SE dosage (Cohort D) were no longer considered responders at D421, this was due to high antibody titers at D0 and they still possessed PRIMVAC specific antibodies.

PRIMVAC isotypic subtypes antibody titres were determined at D0 and 34 days after the third vaccination (D90). Most of the induced antibodies were IgG1, followed by IgG3 in the PRIMVAC/GLA-SE vaccinated volunteers in cohorts C and D (geometric mean 485·7 OD 0·5 [95% CI 351·1-671·9] for IgG1 and 43·6 [95% CI 22·8-83·6] for IgG3 at D90 for PRIMVAC 100 µg) **(Table S7; appendix p17)**. No IgG2 and IgG4 were detected in any of the dosage/adjuvant combinations.

Following PRIMVAC immunization, an increase in the antigen specific IL-2 and IL-5 producing T cell responses was observed at D63 compared to D0 across cohorts (maximum median increase: +36·5 (interquartile range: 18·0-41·0) SFC/10^5 observed for IL-2 in PRIMVAC 50 µg with GLA-SE) **(Figure S2 appendix p 23 and Table S8; appendix p 18)**. The ELISPOT responses tended to be greater with GLA-SE than with the Alhydrogel® adjuvant. No increase in the T cell response was observed with increasing vaccine doses.

To assess the capacity of vaccine-induced antibodies to recognize native VAR2CSA on the IEs surface, sera samples were co-incubated with NF54-CSA, FCR3-CSA and 7G8-CSA purified

IEs. Flow cytometry analysis revealed that the homologous VAR2CSA expressing NF54-CSA IEs were recognized by total serum IgGs from all participants from cohorts C and D as soon as 7 days after the second vaccination (D35) (Cohort D fold changes from baseline at D35 with PRIMVAC 100 µg : 2·29 [95% CI 1·55-3·38] for GLA-SE and 1·52 [95% CI 1·10-2·08] for Alhydrogel) **(Figure 3 and Table S9; appendix p 19)**. Cell surface labelling was confirmed by confocal microscopy **(Figure S3; appendix p 24)**.

At Day 35, NF54-CSA IEs recognition was weaker with samples from cohorts A and B (Cohort B fold changes from baseline: 1·47 [95% CI 0·83-2·59] for GLASE and 1·04 95% CI [0·94-1·15] for Alhydrogel) and barely detectable when considering the 2 arms adjuvanted with Alhydrogel®. Nevertheless, 7 and 30 days after the third vaccination (D63 and D90) all PRIMVAC-vaccinated volunteers possessed antibodies able to react with NF54-CSA IEs, reaching up to a 15-fold increase in Geo. MFI as compared to D0 for participants vaccinated with 20 µg PRIMVAC in combination with GLA-SE. Less recognition was observed against the native VAR2CSA expressed on both FCR3-CSA and 7G8-CSA IEs. Indeed, at D35, none of the PRIMVAC-vaccinated women had relevant antibodies cross-reacting with native VAR2CSA expressed at the surface of FCR3-CSA and 7G8-CSA IEs. Compared to D0, an increased reactivity was observed at D63 and D90 for the highest PRIMVAC dose (Cohort D) adjuvanted with both Alhydrogel® and GLA-SE (up to 1.49-fold increase in Geo. MFI [95% CI 1·19-1·88] in the GLA-SE arm against FCR3-CSA at D63) **(Table S9; appendix p 19)**.

The capability of vaccine-induced antibodies to inhibit the adhesion of VAR2CSA-expressing IEs to the placental receptor CSA was assessed. While inhibition of the interaction of NF54-CSA IEs with CSA was absent or weak in all cohorts at D35, **(Figure 4 and Table S10 appendix p 20),** blocking activity was observed at D63 and D90 for all cohorts. The strongest blocking effect was observed 7 days after the third vaccination (D63) in the PRIMVAC/GLA-

SE cohort D (mean 58·3% inhibition, 95% CI 48·5-68·1). Low or no CSA-binding inhibition was observed on FCR3-CSA or 7G8-CSA IEs expressing heterologous VAR2CSA variants **(Table S10; appendix p 20)**.

All the conclusions regarding the immune responses based on per-protocol analyses **(Table S12, Figures S4-S6, appendix p 25-28)** were consistent with those from the ITT analyses.

DISCUSSION

VAR2CSA-based PM vaccines administered before first pregnancy stand as the main anti-disease strategy to reduce malaria morbidity and mortality[18]. Here, we report the results of a phase Ia/Ib clinical trial, assessing the safety and immunogenicity of the PRIMVAC PM vaccine. PRIMVAC adjuvanted with either Alhydrogel® or GLA-SE presented an acceptable safety profile, as only grade 1 and 2 AEs related to the vaccine were reported. All women vaccinated with PRIMVAC seroconverted after two vaccine doses and antibody titres reached a peak one week after the third vaccination (Day 63). Although PRIMVAC adjuvanted with either Alhydrogel® or GLA-SE was immunogenic in all cohorts, GLA-SE appeared more potent than Alhydrogel® at inducing IgG responses. This higher response is in line with the results obtained in the phase Ia PAMVAC study, which assessed the safety and immunogenicity of a FCR3-VAR2CSA vaccine in malaria naïve volunteers. [20] With respect to each adjuvant, no clear dose-response relationship in terms of IgG response amplitude was observed between the PRIMVAC dose 20 μg and 50 μg in France or between the dose 50 μg and 100 μg in Burkina Faso. However, the geometric mean PRIMVAC-specific antibody titres in the 50 μg cohorts tended to be higher in women from Burkina Faso than in French women. In the context of PM, the emergence of VAR2CSA expressing IEs during natural *P. falciparum* infection is made possible by the appearance of the placenta. Thus, theoretically nulligravid women should not have encountered VAR2CSA expressing IEs to naturally develop an immune response toward the antigen. This is generally reflected by a low VAR2CSA-recognition at baseline. However, out of 50 Burkinabe women, 16 possessed PRIMVAC-specific IgG titres > 100 before vaccination. Field studies have previously reported low levels of anti-VAR2CSA antibodies in children, men and teenage girls.[25-27] Therefore, antibodies to VAR2CSA seems to be acquired, to some extent, outside of the pregnancy context. This could be the consequence of prior exposure to VAR2CSA or to malaria antigens sharing common epitopes with VAR2CSA such

as other PfEMP1 or *P. vivax* Duffy Binding Protein (PvDBP).[28] The higher antibody titres observed in Burkinabe women may thus result from a recall of pre-existing immunological responses.

Highest anti-PRIMVAC IgG levels were seen at the end of vaccination and were accompanied by a release of cytokines by T-cells. Both IL-2 and IL-5 responses tended to be greater with GLA-SE than with Alhydrogel®. Notably, the median of SFC in the IL-2 ELISPOT seems to be lower in the malaria pre-exposed women than in the malaria naive women. IL-2-mediated differentiation of T cells into T_{helper} cells is essential for B cell antibody class switching, and the concomitant secretion of IL-5 will stimulate B cells to produce antibodies. Thus, PRIMVAC seems to orientate the immune system towards a mixed Th1/Th2 response, which is favourable for vaccine development.

VAR2CSA sequence polymorphism represents a major hurdle for PM vaccine development. Efforts have been deployed to design VAR2CSA-based polypeptides able to generate antibodies cross-recognizing semi-conserved antigenic determinants present within different VAR2CSA variants. Our previous pre-clinical studies highlighted that conserved epitopes within VAR2CSA variants were present in restricted numbers and/or were non-immunodominant [13,15,19]. In line with these pre-clinical studies, PRIMVAC was able to generate antibodies reacting with VAR2CSA expressed on the surface of the homologous NF54-CSA strain as soon as 7 days following the second PRIMVAC injection, with a more pronounced effect in both African cohorts, thus indicating that these antibodies are produced at early time-points of the vaccination schedule. However, low cross-recognition of VAR2CSA variants expressed by FCR3-CSA and 7G8-CSA strains was only detected in sera collected at Days 63 and 90 from women who received the 100 ug dose. The weaker recognition amplitude observed for FCR3-CSA and 7G8-CSA IEs than for NF54-CSA suggests that only a restricted number of conserved accessible epitopes exists between the different VAR2CSA variants and/or that

antibodies targeting these conserved epitopes are present in low amounts due to their weak immunogenicity.

The vaccine-induced antibodies were also able to inhibit the adhesion of the homologous VAR2CSA-expressing NF54-CSA IEs to CSA with the higher activity being observed in the 100 µg PRIMVAC dosage adjuvanted with GLA-SE. However, no inhibition of adhesion was observed on the heterologous FCR3-CSA and 7G8-CSA strains. This lack of inhibition is likely the consequence of the low cross-reactivity observed by flow cytometry.

These observations raise an important question regarding the cross-reactive antibody threshold required for protection. PM protection has been correlated with the capacity of antibodies to recognize VAR2CSA-expressing IEs and inhibit their interaction with CSA. However, little is known about other potential protective modes of action and required levels of anti-VAR2CSA antibodies. Furthermore, the importance and the respective role of other antibody-mediated immune effector mechanisms such as Antibody Dependent Cell-mediated cytotoxicity (ADCC) and Antibody Dependent Phagocytosis (ADP) remain unclear.

IgG sub-classes analysis performed 34 days after the last vaccination revealed that most of the induced antibodies were IgG1 and IgG3, both sub-classes being able to interact with most Fc receptors present on immune effector cells. Interestingly, the vaccine-induced antibody response seems to mimic the naturally acquired immune response observed in previous observational studies showing that IgGs from multigravida women reactive to placental parasite isolates were mainly IgG1 and to a lesser extent IgG3 [29,30] and that both sub-classes correlated with the ability of serum or plasma to inhibit adhesion of IEs to CSA [30]. A recent manuscript have shown that IgM is an important functional and long-lived antibody response targeting blood-stage malaria parasites that contributes to malaria immunity [31]. Therefore, it could be interesting in the future to assess IgM vaccine induction.

586 Evaluation of vaccine efficacy remains particularly complex for malaria. To date, the lack of
587 surrogates to predict vaccine-induced protection against PM limits the potential of early clinical
588 trials to provide hints for possible vaccine efficacy. The amplitude of the humoral response
589 resulting from vaccine administration appears today as a promising indicator of potential
590 vaccine efficacy, even though the threshold levels of antibodies required for protection are
591 undetermined. Numerous studies correlated the presence of anti-VAR2CSA antibodies with
592 protection against PM. [2,8,16]
593 PRIMVAC aims at priming the immunity of nulligravid women against placental-type IEs.
594 Interestingly, the 3D7-VAR2CSA PRIMVAC appears here as a vaccine candidate of choice
595 since a recent study suggested that women infected with 3D7-like variants deliver infants with
596 lower birthweight as compared to women infected with FCR3-like variants. [32]
597 In the field, pregnant women are exposed to a variety of parasites harbouring different
598 genotypes and therefore displaying different VAR2CSA variants. The natural boosts of
599 PRIMVAC vaccinated-individuals could then enhance the response against conserved epitopes
600 and consequently accelerate the induction of a durable clinical protection. Therefore, although
601 not planned initially, it seems important to follow the vaccinated Burkinabe women in order to
602 assess the sustainability, longevity and potential expansion of the PRIMVAC induced humoral
603 response in the context of natural exposure and also in the context of future pregnancies. This
604 follow-up could provide some indication whether or not the immune response associated to
605 PRIMVAC could help these women in expanding an immune response likely to protect against
606 PM.
607 In parallel and in order to enhance the PRIMVAC induced immune response, other schedule of
608 immunization, antigen dosage and adjuvant combinations could be assessed in future studies.
609 Alternative strategies could also be explored, such as using other delivery platform, combining

PRIMVAC with other VAR2CSA derived vaccines or using heterologous prime-boost regimens incorporating different variants of the VAR2CSA CSA-binding region.

In conclusion, PRIMVAC presented an acceptable safety profile and was immunogenic in both women never exposed to *P. falciparum* in France and nulligravid women living in a malaria endemic area in Burkina Faso. Seroconversion was observed in all PRIMVAC-vaccinated women and the combination with GLA-SE tends to increase PRIMVAC-specific IgG levels and duration of the response. However, only the higher dosage was able to induce cross-reactive antibodies against other VAR2CSA variants, and no cross-inhibition was observed.

Future studies should seek to increase the level of cross-reactive and cross-inhibitory antibodies. Follow-up of the Burkinabe women vaccinated with PRIMVAC could provide some indication whether or not the immune response associated to PRIMVAC vaccination could help these women in accelerating a clinical protection against PM.

FIGURE LEGENDS

Figure 1: Trial profile. Eighteen volunteers were enrolled in cohorts A and B in France and 50 volunteers were enrolled in cohorts C and D in Burkina Faso. Opening of the cohort B was scheduled if no adverse events following immunization was reported at D7 after the first vaccination in any of the six volunteers of this cohort. Opening of cohorts C and D was realized after safety data review by the DSMB. Among the volunteers, 63 received all three injections of PRIMVAC.

Figure 2. Anti-PRIMVAC IgG ELISA titres. Total IgG response to the PRIMVAC antigen was assessed by Enzyme Linked Immuno-Sorbent Assay (ELISA) at D0, 28, 35, 56, 63, 90, 180 and D421 in cohorts A and B in France (A) and in cohorts C and D in Burkina Faso (B). Data were fit to a 4-parameter sigmoidal curve, and the reciprocal serum dilution at which the optical density was 1·0 (OD1·0) was calculated. Closed circles represent geometric mean antibody titres (GMT) and black bars show 95% confidence intervals (CI).

Figure 3. Immune recognition of native VAR2CSA expressed on the surface of IEs. Erythrocytes infected by the VAR2CSA expressing parasites strain NF54-CSA, FCR3-CSA and 7G8-CSA were incubated with individual serum samples from cohorts A and B (A) and C and D (B) at D0, 35, 63 and D90. Erythrocyte-bound IgGs were detected using an anti-human IgG PE-conjugated antibody. Cells were then subjected to flow cytometry analysis. Results are expressed as the fold-change in geometrical mean fluorescence intensity (PE) between the post-immunization samples and the respective pre-immunization samples. Results are depicted with 95% confidence interval (CI) levels.

Figure 4. Inhibition of IEs binding to the placental receptor CSA by vaccination-induced antibodies. Erythrocytes infected by the autologous parasite strain NF54-CSA were pre-incubated with individual serum samples from cohorts A and B (A) and C and D (B) at D0, 35, 63 and D90. CSA-binding inhibition was assessed by relative quantification of IEs remaining bound to the plate surface after washes. For a given condition, the percentage of inhibition was calculated as follow: [% inhibition = $100-(OD_{post\text{-}immunization}/OD_{pre\text{-}immunization})/100$]. Results are depicted with 95% confidence interval (CI) levels.

CONTRIBUTORS

SBS, Ola and BG were the principal investigators. SBS, LR, AC, ATK, OLe, PL, IN, VB, GR, ELP, AK, EK, HE, FB, ET, NKV, RT, OLa and BG designed the trial. SBS, LR, AC, ATK, SD, JPS, NB, MB, PL, AlO, IN, MoK, DK, AB, SMO, VB, FA, GR, ELP, AK, EK, CR, MyK, LB, AD, NH, IS, AO, HE, Ola, BG acquired and curated the data. AC, ATK, SD, JPS, NB, MB, IN, MoK, DK, AO, FB, ET performed the laboratory experiments. AC, NH, NKV and BG developed the vaccine. LR, CC, and RT performed the statistical analysis. SBS, LR, AC, ATK, CC, CR, SB, JPS, NB, MB, AlO, IN, VB, FB, ET, NKV, RT, OLa and BG interpreted data and results. SBS, LR, AC, CC, RT, OL and BG wrote the first version of the manuscript. All authors critically reviewed and validated the final version of the manuscript.

DECLARATION OF INTERESTS

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REFERENCES

1. World Malaria Report. ISBN: 978 92 4 156565 3. (World Health Organization, Geneva, 2018).
2. Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. *Nature* 2002; **415**(6872): 673-9.
3. Rogerson SJ, Desai M, Mayor A, Sicuri E, Taylor SM, van Eijk AM. Burden, pathology, and costs of malaria in pregnancy: new developments for an old problem. *Lancet Infect Dis* 2018; **18**(4): e107-e18.
4. Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 2007; **7**(2): 93-104.
5. Walker PG, ter Kuile FO, Garske T, Menendez C, Ghani AC. Estimated risk of placental infection and low birthweight attributable to Plasmodium falciparum malaria in Africa in 2010: a modelling study. *Lancet Glob Health* 2014; **2**(8): e460-7.
6. Eisele TP, Larsen DA, Anglewicz PA, et al. Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. *Lancet Infect Dis* 2012; **12**(12): 942-9.
7. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI. Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis. *Lancet Glob Health* 2017; **5**(11): e1101-e12.
8. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. *Nature* 1998; **395**(6705): 851-2.
9. Fried M, Duffy PE. Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta. *Science* 1996; **272**(5267): 1502-4.
10. Salanti A, Staalsoe T, Lavstsen T, et al. Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. *Mol Microbiol* 2003; **49**(1): 179-91.
11. Srivastava A, Gangnard S, Round A, et al. Full-length extracellular region of the var2CSA variant of PfEMP1 is required for specific, high-affinity binding to CSA. *Proc Natl Acad Sci U S A* 2010; **107**(11): 4884-9.
12. Viebig NK, Gamain B, Scheidig C, et al. A single member of the Plasmodium falciparum var multigene family determines cytoadhesion to the placental receptor chondroitin sulphate A. *EMBO Rep* 2005; **6**(8): 775-81.
13. Avril M, Hathaway MJ, Srivastava A, et al. Antibodies to a full-length VAR2CSA immunogen are broadly strain-transcendent but do not cross-inhibit different placental-type parasite isolates. *PLoS One* 2011; **6**(2): e16622.
14. Bigey P, Gnidehou S, Doritchamou J, et al. The NTS-DBL2X region of VAR2CSA induces cross-reactive antibodies that inhibit adhesion of several Plasmodium falciparum isolates to chondroitin sulfate A. *J Infect Dis* 2011; **204**(7): 1125-33.
15. Chene A, Gangnard S, Dechavanne C, et al. Down-selection of the VAR2CSA DBL1-2 expressed in E. coli as a lead antigen for placental malaria vaccine development. *NPJ Vaccines* 2018; **3**: 28.
16. Ndam NT, Denoeud-Ndam L, Doritchamou J, et al. Protective Antibodies against Placental Malaria and Poor Outcomes during Pregnancy, Benin. *Emerg Infect Dis* 2015; **21**(5): 813-23.
17. Babakhanyan A, Leke RG, Salanti A, et al. The antibody response of pregnant Cameroonian women to VAR2CSA ID1-ID2a, a small recombinant protein containing the CSA-binding site. *PLoS One* 2014; **9**(2): e88173.
18. Chene A, Houard S, Nielsen MA, et al. Clinical development of placental malaria vaccines and immunoassays harmonization: a workshop report. *Malar J* 2016; **15**: 476.
19. Chene A, Gangnard S, Guadall A, et al. Preclinical immunogenicity and safety of the cGMP-grade placental malaria vaccine PRIMVAC. *EBioMedicine* 2019.

20. Mordmuller B, Sulyok M, Egger-Adam D, et al. First-in-human, randomized, double-blind clinical trial of differentially adjuvanted PAMVAC, a vaccine candidate to prevent pregnancy-associated malaria. *Clin Infect Dis* 2019.
21. Singh K, Mukherjee P, Shakri AR, et al. Malaria vaccine candidate based on Duffy-binding protein elicits strain transcending functional antibodies in a Phase I trial. *NPJ Vaccines* 2018; **3**: 48.
22. Ishizuka AS, Lyke KE, DeZure A, et al. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nat Med* 2016; **22**(6): 614-23.
23. Bonhoeffer J, Imoukhuede EB, Aldrovandi G, et al. Template protocol for clinical trials investigating vaccines--focus on safety elements. *Vaccine* 2013; **31**(47): 5602-20.
24. Buoen C, Holm S, Thomsen MS. Evaluation of the cohort size in phase I dose escalation trials based on laboratory data. *J Clin Pharmacol* 2003; **43**(5): 470-6.
25. Fodjo BA, Atemnkeng N, Esemu L, et al. Antibody responses to the full-length VAR2CSA and its DBL domains in Cameroonian children and teenagers. *Malar J* 2016; **15**(1): 532.
26. Oleinikov AV, Voronkova VV, Frye IT, et al. A plasma survey using 38 PfEMP1 domains reveals frequent recognition of the Plasmodium falciparum antigen VAR2CSA among young Tanzanian children. *PLoS One* 2012; **7**(1): e31011.
27. Beeson JG, Ndungu F, Persson KE, et al. Antibodies among men and children to placental-binding Plasmodium falciparum-infected erythrocytes that express var2csa. *Am J Trop Med Hyg* 2007; **77**(1): 22-8.
28. Gnidehou S, Mitran CJ, Arango E, et al. Cross-Species Immune Recognition Between Plasmodium vivax Duffy Binding Protein Antibodies and the Plasmodium falciparum Surface Antigen VAR2CSA. *J Infect Dis* 2019; **219**(1): 110-20.
29. Megnekou R, Staalsoe T, Taylor DW, Leke R, Hviid L. Effects of pregnancy and intensity of Plasmodium falciparum transmission on immunoglobulin G subclass responses to variant surface antigens. *Infect Immun* 2005; **73**(7): 4112-8.
30. Elliott SR, Brennan AK, Beeson JG, et al. Placental malaria induces variant-specific antibodies of the cytophilic subtypes immunoglobulin G1 (IgG1) and IgG3 that correlate with adhesion inhibitory activity. *Infect Immun* 2005; **73**(9): 5903-7.
31. Boyle MJ, Chan JA, Handayuni I, et al. IgM in human immunity to Plasmodium falciparum malaria. *Sci Adv* 2019; **5**(9): eaax4489.
32. Patel JC, Hathaway NJ, Parobek CM, et al. Increased risk of low birth weight in women with placental malaria associated with P. falciparum VAR2CSA clade. *Sci Rep* 2017; **7**(1): 7768.

Table 1 –Characteristics of the vaccinated participants

	Cohort A:		**Cohort B:**		**Cohort C:**		**Cohort D:**		**Placebo**	**Total**
	PRIMVAC 20µg		**PRIMVAC 50µg**		**PRIMVAC 50µg**		**PRIMVAC 100µg**			
	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE	Alhydrogel	GLA-SE		
	n=3	n=3	n=5	n=6	n=10	n=10	n=10	n=10	n=10	n=67
Age, years	21	26	30	28	21	20	19	18	19	20
	(20 ; 35)	(24 ; 34)	(27 ; 32)	(27 ; 31)	(20 ; 21)	(19 ; 21)	(18 ; 20)	(18 ; 19)	(18 ; 21)	(18 ; 22)
BMI , kg/m²	22·5	21·1	21·4	23·1	19·9	20·8	21·1	20·0	23·3	21·1
	(21·5 ; 23·6)	(21·0 ; 23·9)	(19·8 ; 25·3)	(20·1 ; 27·7)	(19·0 ; 20·6)	(20·4 ; 21·3)	(20·3 ; 22·2)	(19·5 ; 22·0)	(21·4 ; 25·1)	(19·8 ; 23·7)

Median and interquartile range are shown

Table 2 –Adverse events: number of events (N) and number and proportion of participants with at least one event

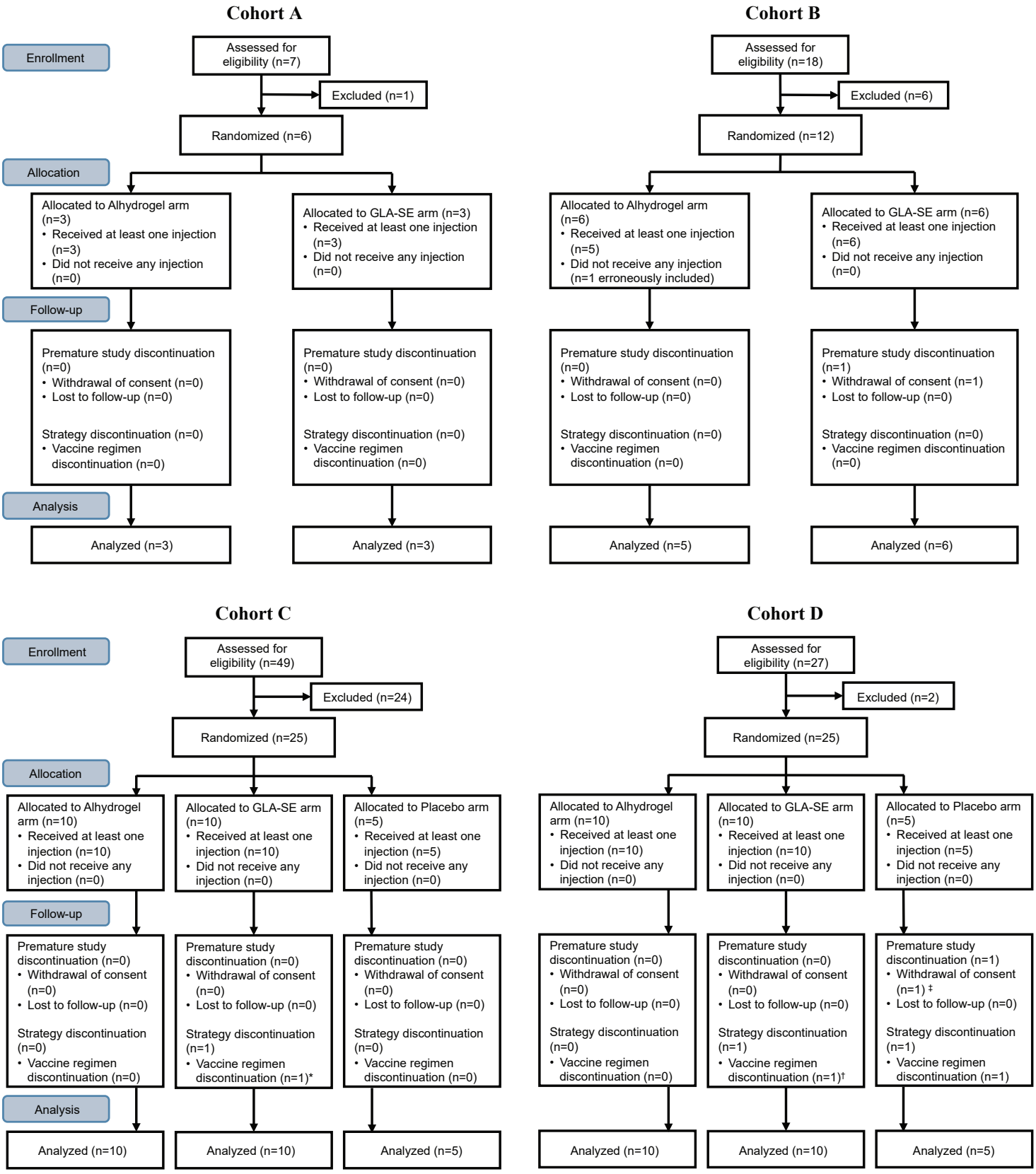
	Cohort A				Cohort B				Cohort C				Cohort D							
	PRIMVAC 20µg				PRIMVAC 50µg				PRIMVAC 50µg				PRIMVAC 100µg				Placebo		Total	
	Alhydrogel (n=3)		GLA-SE (n=3)		Alhydrogel (n=5)		GLA-SE (n=6)		Alhydrogel (n=10)		GLA-SE (n=10)		Alhydrogel (n=10)		GLA-SE (n=10)		(n=10)		(n=67)	
	N	Number of women (%)	N	Number of women (%)	N	Number of women (%)	N=	Number of women (%)	N	Number of women (%)	N	Number of women (%)	N	Number of women (%)	N	Number of women (%)	N	Number of women (%)	N	Number of women (%)
Total adverse events	32	3 (100)	22	3 (100)	57	5 (100)	48	6 (100)	42	10 (100)	35	9 (90)	42	10(100)	33	8(80)	27	9(90)	338	63(94)
AE related to vaccine	25	3 (100)	20	3 (100)	42	5 (100)	42	5 (83·3)	18	6 (60)	10	6 (60)	15	7 (70)	9	4 (40)	5	3 (30)	186	42 (62·7)
After first dose	8	3 (100)	9	3 (100)	16	5 (100)	10	5 (83·3)	5	4 (40)	4	4 (40)	3	3 (30)	3	2 (20)	1	1 (10)	59	30 (44·8)
After second dose	12	3 (100)	5	3 (100)	15	5 (100)	10	5 (83·3)	5	4 (40)	5	4 (40)	8	6 (60)	4	3 (30)	3	2 (20)	67	35 (52·2)
After third dose	5	2 (66·7)	6	3 (100)	11	5 (100)	22	4 (66·7)	8	4 (40)	1	1 (10)	4	4 (40)	2	2 (20)	1	1 (10)	60	26 (38·8)
Grade 1	20	3 (100)	16	3 (100)	22	5 (100)	28	5 (83·3)	14	6 (60)	9	6 (60)	15	7 (70)	8	4 (40)	5	3 (30)	137	42 (62·7)
Grade 2	5	2 (66·7)	4	1 (33·3)	20	4 (80)	14	4 (66·7)	4	2 (20)	1	1 (10)	0	0 (0)	1	1 (10)	0	0 (0)	49	15 (22·4)
Grade 3 and 4	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
Total serious AE	0	0 (0)	0	0 (0)	0	0 (0)	2	2 (33)	10	9 (90)	6	5 (50)	3	3(30)	3	3(30)	8	6(60)	32	28(42)
Serious AE related to vaccine	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)

Table 3 – Number of adverse events related to vaccination by MedDRA System Organ Class (SOC) and Preferred Term (PT)

		Cohort A				Cohort B				Cohort C				Cohort D				Placebo	
		Alhydrogel		GLA-SE		Alhydrogel		GLA-SE		Alhydrogel		GLA-SE		Alhydrogel		GLA-SE			
SOC (and PT), n (%)	**Maximal Grade**	n=25		n=20		n=42		n=42		n=18		n=10		n=15		n=9		n=5	
General disorders and administration site condition Injection site reaction: pain, movement impairment, erythema, induration, swelling, hypoesthesia, haematoma, inflammation, node, oedema, pruritus, asthenia, fatigue, chills	Grade 1	18	(72)	14	(70)	18	(43)	22	(52)	13	(72)	7	(70)	13	(87)	7	(78)	1	(20)
	Grade 2	5	(20)	3	(15)	11	(26)	11	(26)	3	(17)								
Nervous system disorders Headache	Grade 1	2	(8)	1	(5)			1	(2)	1	(6)	1	(10)	1	(7)	1	(11)	4	(80)
	Grade 2			1	(5)	4	(10)									1	(11)		
Musculoskeletal and connective tissue disorders Arthralgia myalgia, dorsalgie	Grade 1					1	(2)	3	(7)										
	Grade 2					2	(5)	2	(5)	1	(6)	1	(10)						
Gastrointestinal disorders Nausea, diarrhea, dyspepsia	Grade 1			1	(5)	2	(5)	2	(5)			1	(10)	1	(7)				
	Grade 2					1	(2)												
Investigations ALAT increased, ASAT increased	Grade 1					1	(2)												
	Grade 2					1	(2)												
Psychiatric disorders Irritability	Grade 2					1	(2)												
Vascular disorders Hot flush	Grade 2							1	(2)										

Version 20·0 of MedDRA was used for coding adverse events

Figure 1



* Pregnancy, D56 (injection visit) not done

† D56 (injection visit) not done

‡ D28 and D56 (injection visit) not done

Figure 2

Figure 2

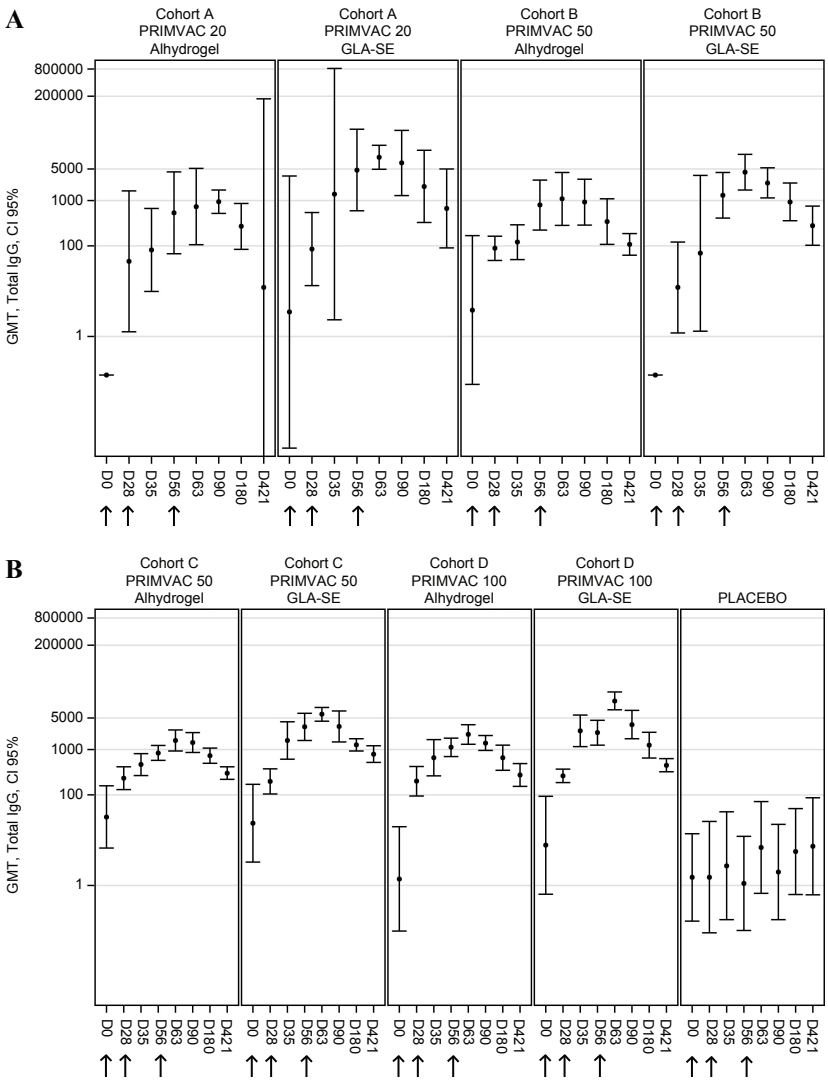


Figure 3

Figure 3

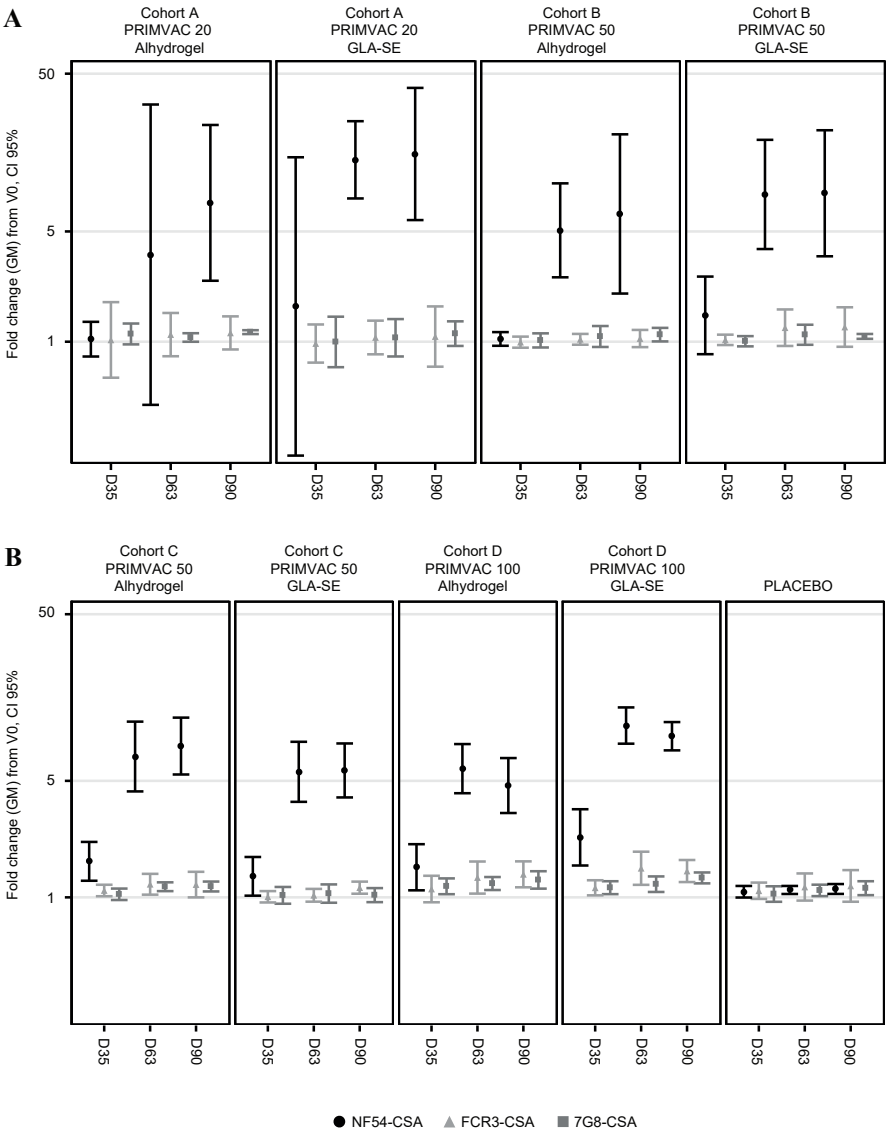


Figure 4

Figure 4